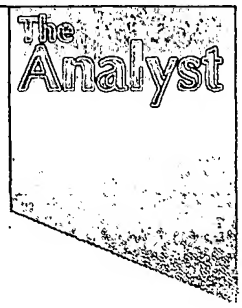


# *In Vitro* Optimisation of a Microdialysis System With Potential for On-line Monitoring of Lactate and Glucose in Biological Samples

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**The optimisation and evaluation of the microdialysis component of a prototype miniaturised total analysis system for application in the continuous monitoring of lactate and glucose is reported. The complete unit comprises a high efficiency microdialysis sampling system, a miniaturised microflow manifold with an integrated biosensor array, together with the hardware and software necessary for controlling the flow parameters and monitoring the sensor signals. Sampling occurs *via* a microdialysis shunt probe which is perfused continuously with a physiological buffered saline solution. The continuous dialysate outflow is presented to the biosensor array, resulting in the appropriate amperometric signals. Aspects of technological significance addressed here include probe membrane size, perfusate flow rate, sample flow rate, temperature change, probe sterilisation procedures, and heparin content of the physiological saline solution employed.**

*Keywords: Microdialysis; lactate; glucose; biosensors; μTAS; on-line monitoring*

Continuous clinical monitoring by on-line chemical analysis using miniaturised instrumentation for real-time measurements represents a significant advance in technology. The concept of a miniaturised total analysis system (μTAS) has been proposed by Manz,[1] consisting of a sampling interface, a microflow manifold and a multi-sensor array for analyte detection. Such a device has potential application in biomedical, biotechnological, industrial, process control and environmental fields. A prototype μTAS device for analysis of glucose and lactate has been developed,[2] and this paper addresses the optimisation of the high efficiency microdialysis sampling component.

The microdialysis technique is a promising sampling tool for continuous biochemical monitoring, allowing determination of concentrations of endogenous compounds upon implantation of a dialysis membrane in the tissue of interest.[3,4] It offers the advantage of a continuous outflow of protein-free aqueous sample (dialysate) which obviates pre-detection sample separation/clean-up steps. Microdialysis probes are available commercially or may be fabricated to requirement, and can be configured as double lumen ('needle type' devices) or single lumen linear probes. Both types were employed here with the majority of the studies based on a linear microdialysis fibre incorporated in a minishunt arrangement.[5]

The majority of human microdialysis studies for glucose/lactate analysis to date have employed subcutaneous sites in healthy volunteers or stable diabetics. However, the probes employed resulted in low sampling efficiency[6–8] or poor time resolution.[9,10] Application of the technique in acute clinical care settings may require intravascular placement for rapid information about acute changes in biochemical parameters, as the interstitial analyte pool may be a poor indicator of systemic analyte status.[11] To our knowledge only one study to date has reported intravascular microdialysis sampling in patients for both analytes.[12]

This report evaluates the sampling efficiency of the microdialysis component of the μTAS using both discrete sample analysis (with a commercial analyser) and analysis using the on-line integrated biosensor.[13] The study allowed selection of a preferred membrane material, size and configuration, thus enabling the development of a prototype on-line glucose and lactate analysis system (with integrated biosensor) for potential patient monitoring[2] using intravascular sampling based on the minishunt arrangement.[5]

## Experimental

### *Materials*

Electrochemical flow measurements were conducted at room temperature in a buffered heparinised modified Dulbeccos solution of pH 7.6, osmolality 300 mOs $kg^{-1}$ $H_2O$, containing 7.54 g $l^{-1}$ NaCl, 0.2 g $l^{-1}$ KCl, 0.2 g $l^{-1}$ $KH_2PO_4$, 1.15 g $l^{-1}$ $K_2HPO_4$, 2.1 g $l^{-1}$ $NaHCO_3$ and 60 mg $l^{-1}$ Enoxaparin. Glucose and lactic acid were purchased from GPR (London, UK) and Sigma (Poole, Dorset, UK), respectively. $KH_2PO_4$, $K_2HPO_4$ and $Na(HCO_3)_2$ of AnalaR grade were purchased from Merck (Poole, Dorset, UK) and NaCl and KCl from Merck (Darmstadt, Germany). Low molecular weight Heparin–Clexane was purchased from Rhone-Poulenc Rorer (Maisons Alfort, France).

Microdialysis probes (CMA/10) and the CMA/100 microperfusion pump were purchased from Carnegie Medicin AB (Stockholm, Sweden). Polyacrylonitrile fibres were obtained from Filtral Haemodialysis (Hospal AN-69HF; Lyon, France) cartridges. Aminocellulose fibres were initially obtained from opened Allwall GF Haemodialysis cartridge (Gambro, Hemophan; Hechingen, Germany) and later as raw fibres from Akzo Membra (Wuppertal, Germany). Clinical PVC tubing (141.00 and 220.10) was purchased from Vygon, Dublin, Ireland. Transport tubing from microdialysis fibre to inlet/outlet was polyimide-coated fused silica capillary (TSP 100170) from PolymicroTechnologies, (Phoenix, AZ, USA) and inlet/outlet tubing was 16G polyethylene catheter (Portex; Hythe, Kent, UK) glued with UV curing PVC bonding adhesive (Loctite 330; Tallaght, Dublin). Inert FEP tubing attached to the probe inlet/outlet was suitable for liquid flow to and from the probe. Glass capillaries (Clinitubes; from Radiometer, Copenhagen, Denmark) were employed for dialysate collection. A Braun percutaneous nephrostomy needle, 19G, 20 cm long was used for introduction of dialysis fibre into the sampling tube.

### *Experimental Setup*

The complete microdialysis system (Fig. 1) includes a microinjection pump (CMA/100) for delivery of the perfusion fluid, 3 × 3 way valves for diversion of flow during sensor calibration or microdialysis, a simulated vascular circuit incorporating a peristaltic pump, the microflow stack/integrated biosensor for analyte detection[2,13] together with interface to LapTop PC.

Commercial co-axial needle type microdialysis probes (CMA/10) of molecular weight cut-off 20 000 Da, 24 mm total shaft length with a polycarbonate membrane diameter of 0.5 mm and length 16 mm were employed in the initial studies. The majority of the work involved aminocellulose or polyacrylonitrile dialysis membranes in the minishunt arrangement (Fig. 2). Short microdialysis outflow lines were used to reduce back pressure and dispersion of the sample due to longitudinal dispersion. Preparation of the microdialysis minishunt probes employed has been described previously.[5]

The detection system employed consisted of a microflow manifold, comprising a stack of photolithographically etched silicon wafers[14] which presented either calibrant or dialysate sample to the integrated biosensor[13,15] (at the base of the stack). The amperometric biosensor required a separate four-channel potentiostat for application of the potential (500 mV *versus* Ag/AgCl). Data acquisition was achieved using LabView 3.0 Software (National Instruments, TX, USA).

The YSI 2300 STAT-PLUS glucose and lactate analyser (Yellow Springs Instruments, OH, USA),[16] was employed for some of the microdialysis *in vitro* evaluation studies and used as a comparison method for biological sample (serum or plasma) analysis.

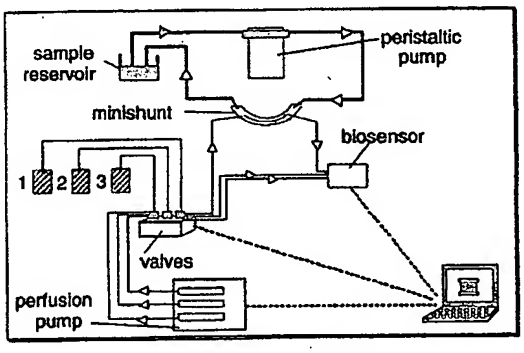


Fig. 1 Experimental setup showing microdialysis perfusion pump, 3 × 3 way valves and biosensor signal acquisition under computer control; a simulated vascular circuit incorporating a peristaltic pump for pumping blood/serum through the minishunt; microdialysis perfusion pump pumps perfusion buffer through the inner fibre of the minishunt at 3.0 μl $min^{-1}$ in countercurrent direction. Reservoirs 1, 2 and 3 contain buffer, low (0.2 mM and 1 mM) lactate and glucose standard and high (2 mM and 10 mM) lactate and glucose standard, respectively.

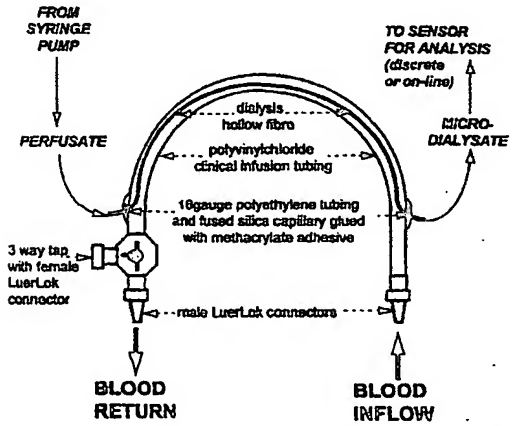


Fig. 2 Linear microdialysis probe in the shunt arrangement. Reproduced with permission of the editor of *Technol. Health Care.*

### *Procedures*

The measure of dialysis efficiency employed is percentage relative recovery (*%RR*). This is determined by placing a microdialysis probe in a standard lactate or glucose solution, and perfusing the probe with modified Dulbeccos buffer at a certain flow rate. The fluid leaving the probe (the dialysate), containing the analyte of interest, is collected and analysed. The analyte concentration in the dialysate is then compared with that in the original standard solution and expressed as a percentage. This value represents the efficiency of the membrane to dialyse analyte from the solution to which it is exposed.

$$\%RR = \frac{\text{Dialysate [Analyte] (mM)} \times 100\%}{\text{Sample [Analyte] (mM)}}$$

The factors affecting microdialysis efficiency *in vitro* were evaluated using both commercial (CMA/10) probes; 16 mm membrane length and custom made linear probes, (Fig. 2) in the minishunt arrangement with simulated vascular circuit shown in Fig. 1. The peristaltic pump was used to pump calibrants (2 mM L-lactate and 8 mM D-glucose) through the minishunt microdialysis probe while the microdialysis pump perfused the inner fibre with modified Dulbeccos buffer in the countercurrent direction. The resultant microdialysate could be collected for serial discrete analysis using the YSI STAT autoanalyser or presented to the on-line integrated biosensor element for quantitative analysis. For the former, capillary tubes (70 μl internal volume) were employed for collection of the dialysate sample at the minishunt probe outlet.

In order to carry out *in vitro* calibration of the microdialysis system/integrated biosensor, the microdialysis probe (CMA/10) or linear minishunt probe was exposed to standard solutions of glucose/ lactate or plasma/serum samples *via* the peristaltic pump and *in vitro* percentage relative recovery (*%RR*) determined. Upon application of the electrode potential ($E_{app}$ = 500 mV *versus* Ag/AgCl) to the integrated biosensor the background amperometric current was allowed to decay prior to analysis, until such time as a 1–2 nA background was achieved.

A two-point calibration of the biosensor itself was achieved by diversion of buffer from reservoir 1 (Fig. 1) to waste and by switching the appropriate valve such that low/high calibrants from reservoirs 2/3 could be pumped through the integrated biosensor. The resulting current signals were stored and the slope (sensitivity nA $mM^{-1}$) calculated.

## Results and Discussion

Microdialysis efficiency is influenced by a number of factors, both physical and physiological. Relevant parameters addressed here include microdialysis perfusion pump flow rate, membrane composition and size, peristaltic pump flow rate, temperature, heparin levels in the modified Dulbeccos buffer, the effect of sterilisation on microdialysis efficiency, reproducibility and stability of measurements by the overall system.

Fig. 3 shows the effects of perfusion flow rate on the relative recovery of lactate and glucose using the commercial CMA/10 probe (active membrane length 16 mm, area 2.51 $mm^2$) (immersed in stirred calibrants). Studies were performed using the integrated biosensor for dialysate analysis and *%RR* calculations. Similar recoveries were found for both analytes,

up to 93% at 1.5 μl $min^{-1}$. Recovery was found to decrease from 93 ± 0.79% to 13.57 ± 0.79% (L-lactate) and from 93 ± 0.79% to 9.97 ± 0.25% (D- glucose) over the range 1.0–20 μl $min^{-1}$. If a longer probe is employed (increased surface area) the curve would be expected to level off at a higher recovery. The effects of perfusion flow rate on the relative recovery of lactate and glucose using the aminocellulose fibre 100 mm length (AC-100) is also shown in Fig. 3. The mean %*RR* of lactate and glucose approached 100% at flow rates less than ≈ 4.0 μl $min^{-1}$ and decreased to 40% at 20 μl $min^{-1}$. All subsequent experiments were carried out at a constant flow rate of 3.0 μl $min^{-1}$. One of the main reasons for employing the minishunt arrangement was to allow for use of probes with longer membrane lengths as compared to the commercially available concentric probes of limited length, and thus increase dialysis efficiency (%*RR*).

Investigation of the effects of external fluid flow on the recovery of analytes through the microdialysis membrane was carried out to see whether potential alteration in blood flow past the active membrane would affect microdialysis efficiency. Glucose (8 mM) and lactate (2 mM) standard solutions were pumped *via* the peristaltic pump (Fig. 1) at different rates through the minishunt past the fibre (aminocellulose 100 mm) in a countercurrent direction to the perfusion pump flow (maintained constant at 3.0 μl $min^{-1}$). The effect of increasing the peristaltic pump flow rate (0.2–20 ml $min^{-1}$) on dialysis efficiency was assessed by determining the percentage relative recovery of analyte [Figs. 4(*a*) and (*b*)], using the YSI analyser for dialysate sample analysis ($n$ = 6). A constant flow rate of 3.0 ml $min^{-1}$ was chosen for future experiments.

The performance of dialysis membranes of various size, configuration and composition were assessed using the YSI bench analyser for dialysate analysis. The effect of membrane surface area employed in the minishunt on microdialysis efficiency for both aminocellulose (AC) and polyacrylonitrile (PAN) membranes was investigated. At fixed perfusion rates of 3.0 μl $min^{-1}$ percentage relative recoveries of both lactate and glucose from aqueous standards (2 and 8 mM, respectively) pumped through the shunt at 3.0 ml $min^{-1}$ reached an optimum 100% after ≈ 60 $mm^2$ for both membranes and analytes [Figs. 5(*a*) and (*b*)].

The temperature effect on membrane dialysis efficiency during microdialysis of human serum samples was determined *in vitro* (Table 1), using the integrated biosensor for dialysate analysis. Microdialysis was performed with serum samples incubated at different temperatures in the range 17.5–40° C. Over this range efficiency increased by 0.33% and 0.54% per degree Celsius for lactate and glucose, respectively. Therefore over the physiological range (34–40° C) no appreciable increase was observed. This study examines the effect of temperature on the dialysis process only. The temperature sensitivity of the catalase covered integrated biosensors was found to be 2.3% per degree Celsius. Dialysis takes place as perfusion fluid at room temperature is pumped through the fibre at 3 μl $min^{-1}$, while sample is pumped through the shunt, past the fibre, at a rate of 3.0 ml $min^{-1}$. The heat transfer to the dialysate as the serum sample is pumped past the fibre should not significantly raise the temperature of the dialysate itself and the heat will dissipate before reaching the biosensor such that analysis is carried out at room temperature.

A stability study for the aminocellulose (100 mm) minishunt probe was performed in 10% human serum albumin (HSA) (spiked with 2.0 mM L-lactate and 8.0 mM D-glucose). Continuous microdialysis was performed over a three-day period with intermittent YSI sampling and analysis, in order to investigate the effects of protein build up/fouling on the %*RR* of analyte across the membrane. Results showed little diminution in microdialysis efficiency over the period. A percentage

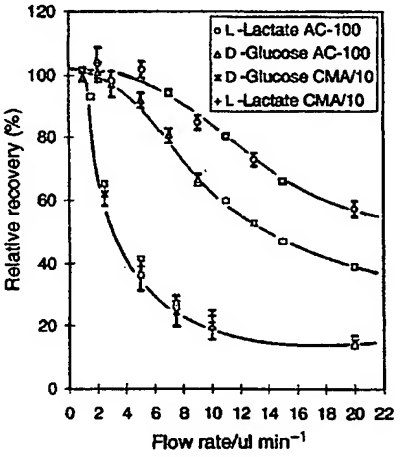


Fig. 3 Effect of perfusion pump flow rate on percentage relative recovery of lactate and glucose from aqueous standard solutions using both the concentric commercial probe (CMA/10), mean ± $s$ ($n$ = 3), and the aminocellulose 100 mm probe in minishunt configuration, mean ± $s$ ($n$ = 6). Peristaltic pump flow rate 3.0 ml $min^{-1}$, $E_{app}$ = 500 mV *versus* Ag/AgCl.

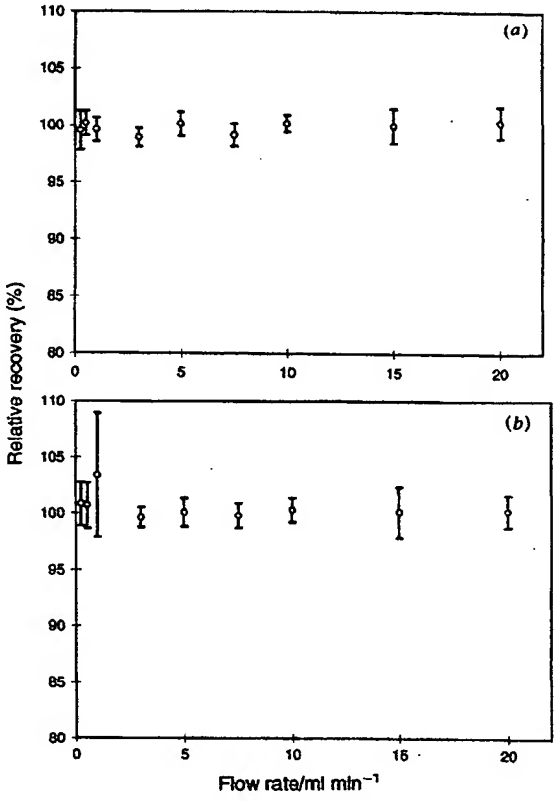


Fig. 4 Effect of peristatic pump (sample) flow rate on dialysis efficiency of minishunt (aminocellulose 100 mm) for both glucose (*a*) and lactate (*b*). Microdialysis carried out at room temperature in aqueous standards of 2 and 8 mM lactate and glucose, respectively. Percentage relative recovery calculated with respect to the absolute concentration as determined by the YSI bench analyser. All other conditions as Fig. 3. Mean ± $s$ plotted ($n$ = 3).

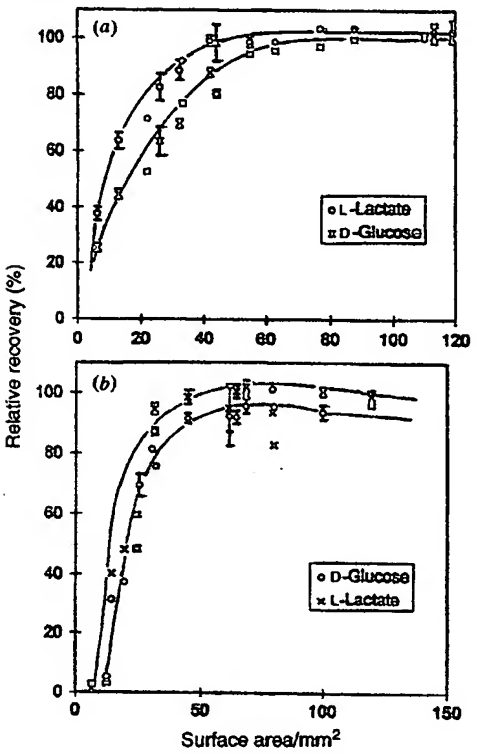


Fig. 5 (*a*) Effect of membrane internal surface area on percentage relative recovery for both analytes using aminocellulose membrane in minishunt arrangement. Experiments carried out at room temperature in aqueous standards of 2 and 8 mM lactate and glucose, respectively. Percentage relative recovery calculated with respect to the absolute concentration as determined by the YSI bench analyser. Perfusion flow rate 3.0 μl $min^{-1}$ with aqueous solutions pumped through the shunt at 3.0 ml $min^{-1}$. Mean plotted ± *s* (*n* = 6). (*b*) Conditions as (*a*) except polyacrylonitrile membrane material. Mean plotted ± *s* (*n* = 6).

Table 1. Effect of serum sample temperature on minishunt (aminocellulose 100 mm) microdialysis efficiency*

| Temperature/°C | *n* | *%RR* D-glucose (mean ± *s*) | *%RR* L-lactase (mean ± *s*) |
|---|---|---|---|
| 17.5 | 5 | 92.02 ± 6.4 | 95.32 ± 2.9 |
| 25 | 5 | 92.77 ± 15.6 | 92.44 ± 2.9 |
| 31 | 5 | 91.58 ± 8.45 | 92.45 ± 19.58 |
| 38 | 5 | 97.28 ± 14.3 | 98.96 ± 17.14 |
| 45 | 5 | 100.8 ± 1.0 | 107.1 ± 1.8 |

* Perfusion flow rate of 3.0 μl $min^{-1}$; peristaltic pump flow rate 3 ml $min^{-1}$; integrated biosensor used for dialysate analysis.

change of 0.04% and 0.26% per hour over a 64 hour period was observed for lactate and glucose, respectively. RSD were 2.5% and 6.7% for *n* = 18 measurements over the time period investigated.

The probes employed in initial studies were not sterile and would require sterilisation before application *in vivo*. Probes were therefore subjected to two sterilisation methods and microdialysis efficiency was assessed pre- and post-sterilisation using probes made from raw aminocellulose (100 mm length) fibres. The sterilisation methods employed were (i) purging with ethylene oxide gas and (ii) gamma irradiation. The microdialysis efficiency of similar control probes not subjected to either method was measured initially and following a time interval identical to that required by the sterilisation procedures. Mean values for the observed change in microdialysis shunt performance are shown in Table 2. No marked difference in *%RR* was evident between groups. Mean percentage difference between relative recovery pre- and post-sterilisation using ethylene oxide sterilisation was −1.6% and −1.7% for glucose and lactate, respectively. Pre- and post-mean changes in percentage relative recovery for gamma irradiation were −0.7 % and −0.6% for glucose and lactate. These differences were found not to be of an order of magnitude to result in any practical significance. Mean change in control probes not subjected to sterilisation procedures but re-tested was +1.3% and −0.1% for glucose and lactate, respectively. The microdialysis experiments were performed using pumped glucose and lactate solutions (8 mM and 2 mM, respectively) at a peristaltic pump flow rate of 3.0 ml $min^{-1}$. Glucose and lactate dialysate concentrations were measured using the YSI STAT analyser.

The ethylene oxide sterilisation method could be used, but gamma irradiation would be the method of choice for safety reasons in clinical application. Sterility is maintained (if the probe is sterilised) even when using a non-sterile perfusion medium, due to the fact that micro-organisms do not penetrate the membrane.

The effect of low molecular weight heparin on the dialysis efficiency was then assessed *in vitro* by measuring the *%RR* of standard solutions of lactate (2 mM) and glucose (8 mM) across the aminocellulose (100 mm) minishunt membrane with increasing concentration of heparin in the perfusion medium. The percentage changes in relative recovery shown (Table 3) make no appreciable difference in practical terms. The perfusion buffer pH and osmolality were measured with increasing concentration of Enoxaparin, pH 7.84, 299 mOs $kg^{-1}$ $H_2O$ (20 mg $l^{-1}$ Enoxaparin), pH 8.37, 303 mOs $kg^{-1}$ $H_2O$ (60 mg $l^{-1}$ Enoxaparin), pH 8.75, 309 mOs $kg^{-1}$ $H_2O$ (100 mg $l^{-1}$ Enoxaparin). Enoxaparin (60 mg $l^{-1}$) was chosen for further experiments.

Overall average *%RR* for glucose in serum using the aminocellulose (100 mm) minishunt at a perfusion flow rate of 3.0 μl $min^{-1}$ with a peristaltic pump flow rate of 3.0 ml $min^{-1}$ was found to be 97.7 ± 6.1% (*n* = 14) [YSI Dialysate (mM)/YSI Serum (mM)] and 98.3 ± 5.3% (*n* = 14) [integrated biosensor (mM)/YSI Serum (mM)]. Percentage relative recovery for lactate under the same conditions was 104.8% ± 4.6% [YSI Dialysate

Table 2 Effect of two sterilisation techniques on *%RR* of minishunt (aminocellulose 100 mm) membranes*

| Sterilisation method | *%RR* Glucose (basal) | *%RR* Glucose (retested) | Change in mean *%RR* | *%RR* Lactate (basal) | *%RR* Lactate (retested) | Change in mean *%RR* |
|---|---|---|---|---|---|---|
| EtO | 100.5 ± 0.7 | 98.8 ± 2.1 | −1.6 | 101.7 ± 1.4 | 100.0 ± 0.9 | −1.7 |
| γ-Radiation | 100.0 ± 0.5 | 99.8 ± 2.8 | −0.7 | 100.5 ± 1.0 | 99.9 ± 0.7 | −0.6 |
| Control | 99.9 ± 1.3 | 101.2 ± 1.2 | +1.3 | 99.8 ± 2.2 | 99.7 ± 2.2 | −0.1 |

* Values given are percentages (mean ± *s*, *n* = 6) before and after sterilisation. Microdialysis was performed in aqueous calibrants at analyte concentrations of ≈ 2 mM L-lactate and 8 mM D-glucose, using the YSI for analysis.

(mM)/YSI Serum (mM)] and 109% ± 9.8% [integrated biosensor (mM)/YSI Serum (mM)].

The performance of any given microdialysis probe in terms of relative recovery requires initial characterisation, and must be evaluated together with investigation of recalibration requirements if accuracy is to be maintained for the duration of the monitoring period. As the simulated vascular circuit and minishunt allowed 100% microdialysis efficiency to be achieved *in vitro,* this obviates absolute requirement for microdialysis probe function calibration and eliminates the requirement for correction factors in data analysis. However, independent sensor calibration during the course of the monitoring period will always be a requirement.

This paper reports *in vitro* optimisation of a high efficiency microdialysis probe which incorporates a membrane surface area currently unavailable commercially. Features such as the ability to calibrate the minishunt externally, accessibility to the probe for visual inspection, replacement or repair, and the fact that this approach allows for a minimally invasive/ implant free sampling approach, all aid its application in intravascular sampling.

Following optimisation of performance of the various components of the complete system it was applied as a total chemical analysis system in an *in vivo* application.[2]

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**Table 3** Change in %*RR* for minishunt (aminocellulose −100 mm) with increasing levels of Enoxaparin in perfusion medium*

| Enoxaparin concentration/ mg l$^{-1}$ | %*RR* D-glucose | Change in %*RR* D-glucose | %*RR* L-lactate | Change in %*RR* L-lactate |
|---|---|---|---|---|
| 20 | 101 ± 2.5 | — | 101.76 ± 0.3 | — |
| 60 | 104.1 ± 2.7 | +3.1 ± 4.0 | 100 ± 3.6 | −1.8 ± 3.2 |
| 100 | 102.7 ± 1.9 | +1.7 ± 1.9 | 100.5 ± 3.2 | −1.3 ± 4.3 |

* Mean ± *s* (*n* = 3). Other conditions as in Table 2.

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